(a) generating a DNA fragment[(s)] which expresses a molecule which enhances the antigen presentation function of an APC;

(b) distributing said DNA fragment[(s)] on a particle surface, resulting in a particulate polynucleotide;

(c) delivering said particulate polynucleotide to the cytoplasm of [a target] an antigen presenting cell of a mammalian host *in vitro*, such that said [expressed] antigen presentation enhancing protein [or proteins] is expressed [at biologically effective levels]; and,

(d) inoculating said mammalian host with said [target] antigen presenting cell by direct injection.

REMARKS

The Final Rejection issued in the parent case, U.S. Serial No. 08/535,556, ("Final Rejection") indicated that the specification was objected to under 35 U.S.C. § 112, first paragraph. Applicants' previous arguments were deemed not convincing, as the Final Rejection indicated that one skilled in the art could not use the claimed invention in a method of genetic immunization without undue experimentation. This objection is respectfully traversed.

The thrust of the Final Rejection is that Applicants have not enabled the invention. More specifically, the Final Rejection indicates that Applicants have failed to demonstrate gene transfer and expression of any other antigenic proteins. It is further alleged that no evidence is provided to extrapolate to other tumor models, other tumor rejection antigens, or to results obtained in a human therapeutic procedure.

As stated in Applicants' previous response, Applicants' original work utilized a known mouse tumor model, accepted by those skilled in the art for the study of immunology. In addition, Applicants selected the OVA antigen because it is representative of all other antigens, and it is the same as many naturally occurring tumor antigens. Those skilled in the art recognize that OVA is appropriate for the type of studies undertaken by Applicants. Numerous refereed articles discuss the use of mouse tumor models and/or OVA antigen, demonstrating that Applicants' methodologies would be accepted by those skilled in the art as providing representative and reliable data. This data provides a reasonable expectation of

success in repeating the methodologies claimed by Applicants using different tumors and/or different antigens without undue experimentation. For example, Mayordomo et al. ("Bone Marrow-Derived Dendritic Cells Pulsed with Synthetic Tumour Peptides Elicit Protective and Therapeutic Antitumour Immunity", *Nature Medicine*, Vol. 1, No. 12, pp. 1297-1302, (December 1995)) used C57BL/6 mice and melanoma tumors in conjunction with OVA antigen in immunization studies. Porgador and Gilboa ("Bone Marrow-Generated Dendritic Cells Pulsed With a Class I-Restricted Peptide are Potent Inducers of Cytotoxic T Lymphocytes", *J. Exp. Med.*, Vol. 182, pp. 255-260 (July 1995)) also used C57BL/6 mice and the OVA antigen in their study of elicitation of CTL responses. (See, also, Falo et al., "Targeting Antigen into the Phagocytic Pathway *In Vivo* Induces Protective Tumour Immunity", *Nature Medicine*, Vol. 1, No. 7, pp. 649-653 (July 1995), use of C57BL/6 mice, B16 melanoma model and OVA antigen; Celluzzi et al., "Peptide-Pulsed Dendritic Cells Induce Antigen-Specific, CTL-Mediated Protective Tumor Immunity", *J. Exp. Med.*, Vol. 183, pp. 283-287 (January 1996), use of C57BL/6 mice, B16 melanoma and OVA antigen; Celluzzi and Falo, "Epidermal Dendritic Cells Induce Potent Antigen-Specific CTL-Mediated Immunity", *The Journal of Investigative Dermatology*, Vol. 108, No. 5, pp. 716-720 (May 1997), use of C57BL/6 mice, B16 melanoma and OVA antigen; and Condon et al., "DNA-Based Immunization by *In Vivo* Transfection of Dendritic Cells", *Nature Medicine*, Vol. 2, No. 10, pp. 1122-1128 (October 1996), use of C57BL/6 mice, B16 melanoma and OVA antigen. Copies of cited articles are enclosed.)

Moreover, Applicants have submitted evidence that antigens other than OVA work in the methods of the present invention. The Final Rejection acknowledged the data presented in the Declaration submitted with the December 6, 1996 Amendment, which demonstrated that the reporter gene encoding GFP, also works in the manner claimed by Applicants. Notwithstanding the acceptance of this additional data, the Final Rejection still states that "the use of two antigens does not sufficiently enable all antigens." Applicants respectfully request guidance on the number of antigens that *would* sufficiently enable all antigens, and further request citation to any statutory or regulatory requirements or case law which support this number.

Regardless of the number, Applicants submit that in light of the mechanism through which their invention is believed to operate, the demonstration of even one antigen is sufficient to support patentability. The inherent properties of antigen presenting cells (APCs) are used to accomplish the claimed immunization methods. The claims have been amended to specifically recite use of APCs in the methods of the present invention. The Final Rejection comments that because other antigens are not well known or characterized, "it is unpredictable that they will elicit an immune response" and that "it is well known in the immunology art that the expression of antigens does not guarantee an immune response." What is well known in the immunology art is that antigens are defined on the basis of their immunogenicity; that is--an antigen by definition has the ability to be recognized by and stimulate T-cells. Furthermore, the function of APCs is to present antigens to T-cells. Because of this communication, one skilled in the art would accept that immunogenic antigens presented by an APC would elicit an immune response. Moreover, one skilled in the art would accept that immunogenic antigens taken up by an APC would be presented by that APC. Thus, one skilled in the art would accept that introduction of an antigen to the cytoplasm of an APC would result in expression of the antigenic protein or protein fragment on the membrane surface of the APC through the MCH class I pathway such that an immune response is elicited. This is precisely the point of the invention, which the comments quoted above from the Final Rejection seem to miss.

Despite the clear evidence that the Applicants' method functions in the manner claimed and the acknowledgement that this has been demonstrated using the mouse OVA/B16 model, the Final Rejection once again points to the "unpredictable" nature of gene therapy, including short term expression of a therapeutic protein. It is well known in the art that short term expression is appropriate for an immunological methodology. For example, U.S. Patent No. 5,589,466, directed to a method for inducing a protective immune response, clearly states that short term expression, or "transient gene therapy" is exactly what is required to induce an immune response. Just as in the '466 patent, Applicants do not rely on long term expression or integration of the introduced DNA into the genome of the host, but rather require only short term expression. Applicants have demonstrated that the expression offered by the APC according to the methods of

the present invention is sufficient to elicit the claimed immune response. Again, the level of expression required for "gene therapy" and the level of expression required for an immunological response is not necessarily the same. The art recognizes the viability of DNA vaccination. In the enclosed article from "Vaccine Weekly" December 15, 1997, DNA vaccination is reported as potentially revolutionizing human immunization and that such vaccination methodologies offer advantages over classic vaccine methods. Applicants recognized these advantages at the time of filing a patent application on the present invention. (See, also, Xiang et al., "Genetic Vaccines - A Revolution in Vaccinology?", *Springer Semin. Immunopathol.*, Vol. 19, pp. 257-268 (1997) and Raz, "Introduction: Gene Vaccination, Current Concepts and Future Directions", *Springer Semin. Immunopathol.*, Vol. 19, pp. 131-137 (1997), copies of which are enclosed.)

The Final Rejection cites once again to the Orkin reference for the proposition that there has not been demonstrated efficacy in humans using gene therapy strategies. Lack of human data is a recurrent theme throughout the Final Rejection, and is inappropriate. For example, the Final Rejection is critical of Applicants' alleged failure to demonstrate "the in vivo production of antigenic proteins in cells of a human . . . ." Although Applicants have not demonstrated the efficacy of their methods using a human model, which demonstration is *clearly* unnecessary for patentability, Applicants do present evidence that their methods have a reasonable expectation of success in a human; additional evidence for this point is submitted in the attached second Declaration, and is discussed below.

Applicants have shown that the methods of the present invention are effective in a mouse model using two different antigens, the OVA antigen and the GFP reporter gene. As discussed in the second Declaration of Lou Falo, attached, APCs are also capable of uptake and expression of yet a third antigen, the lacZ reporter gene. Applicants used human skin in the lacZ testing. Thus, Applicants have demonstrated that human antigen presenting cells will express antigens *in vivo*. Similar expression was seen with mouse APCs *in vivo*, which expression was then shown to elicit the claimed immunological response. That the human cells function in the same manner of mouse cells gives one skilled in the art a reasonable expectation that the *in vivo* immunological response elicited in the mouse model would also occur in a human.

In addition, other investigators have demonstrated the efficacy of Applicants' methods utilizing different animal models and different antigens. For example, the enclosed Tüting et al. reference ("Autologous Human Monocyte-Derived Dendritic Cells Genetically Modified to Express Melanoma Antigens Elicit Primary Cytotoxic T Cell Responses In Vitro: Enhancement by Cotransfection of Genes Encoding the Th1-Biasing Cytokines IL-12 and IFN-α^1", *The Journal of Immunology*, Vol. 160, pp. 1139-1147 (1998)) utilized DNA based immunization strategies similar, if not identical, to those claimed by Applicants with several different antigens. Significantly, the tests were run using human dendritic cells and human tumor antigens. The results verify that Applicants' methodologies will work with other antigens in human cells. Similar methodologies were also employed by Kim and Weiner ("DNA Gene Vaccination for HIV", *Springer Semin. Immunopathol.*, Vol. 19, pp. 175-194 (1997)). That article reports the use of DNA vaccination for HIV in primates.

Along this line, the Final Rejection further disagrees with Applicants' assertion that "it is well established that animal models can be used to demonstrate efficacy for other mammalian hosts, including humans." It is beyond question that human examples are not required for patentability. For example, Applicants are aware of at least four issued U.S. patents directed to immunology, none of which show results obtained from humans. U.S. Patent No. 5,643,578, to Robinson et al., does not mention any example utilizing human data. U.S. Patent Nos. 5,580,859, 5,589,466 and 5,593,972 offer human examples, but they are only hypothetical examples; there is no actual *in vivo* human data presented in any of these issued patents. The '859 and '466 patents do offer examples utilizing human cells, but these are cell cultures, and not *in vivo* data. This is akin to the presentation made by Applicants in the attached Declaration.

The Final Rejection goes on to say that "there is no well-known, art-recognized, predictive animal model which exists for demonstrating the efficacy or therapeutic effect of Applicants' claimed method." This comment is similarly without basis. If there was a well known, art-recognized predictive animal model which disclosed Applicants' claimed method, then novelty would clearly be an issue. It is anomalous to suggest that there must be an art-recognized model establishing Applicants' claimed method in light of the novelty requirement of

patentability. In any event, Applicants have used a mouse model well accepted in the art of immunology in establishing the efficacy of the claimed methods. All of the four patents cited in the preceding paragraph rely on animal models to support patentability, and all four rely on the mouse model. Applicants' evidence, therefore, would clearly be accepted by those skilled in the art.

The Final Rejection continues that there are no examples or teachings demonstrating that the methods of the present invention would be therapeutically beneficial to a human subject, and that Applicants fail to show regulation of a therapeutic gene under physiological conditions. As extensively discussed above, Applicants have provided sufficient and art accepted evidence supporting their methods. In addition, the mouse model represents physiological conditions. Finally, the concern about "overexpression" raised in the Final Rejection is misplaced. The DNA expression required to elicit an immune response is transient and could not result in overexpression or deleterious effects.

Notwithstanding the criticisms of the examples presented by Applicants in the specification as filed and the first Declaration, the Final Rejection concedes that Applicants have in fact demonstrated delivery of a specific antigen to a mouse, expression of the antigen in the mouse, and rejection from a tumor challenge in the mouse. The Final Rejection detracts from these showings, by stating that gene transfer expression is multi-factorial and that things such as the specific promoter, enhancer, coding sequences, etc. have not been shown. Applicants submit that one skilled in the art would be able to evaluate all of these factors and determine the relevant promoters, enhancers and the like for the particular antigen being used. There is no requirement that Applicants need to show that each and every construct under the sun works in order to enable the invention. For example, the '972 patent discussed above refers to DNA constructs in the delivery of a DNA sequence but does not appear to teach every potential construct available to the skilled artisan. Furthermore, the Tüting et al. and the Kim and Weiner articles show that it is within the skill of the artisan to repeat Applicants' methodologies using a variety of different constructs. Applicants have demonstrated that two distinctly different constructs work in the methods of the present invention, namely the construct containing OVA and the construct containing GFP. It would be an undue burden for Applicants to have to show that

an undefined number of other constructs also work. Again, Applicants request identification of the number of constructs that would be deemed sufficient to illustrate the present invention, and a citation to a statutory or regulatory requirement or case law which sets forth this number.

The Final Rejection is also critical of Applicants' alleged failure to provide guidance on how to make and use a pharmaceutical composition. As discussed in the previous Amendment, Applicants clearly have shown how the particulate polynucleotide can be introduced to the mammalian host. See, for example, Examples Sections 7 and 8 of the specification. According to the Final Rejection, the specification also fails to teach a route and time course of administration, and again cites to the Orkin reference for the "unpredictability" in the art. Again, however, the Orkin reference refers to long term gene expression, and not the short term gene expression required by the present invention. The citation to Orkin further ignores the fact that patents for gene therapy have issued. In any event, the examples clearly show the route of administration. The time course of administration would be dependent upon numerous variables, such as the reaction of the host, the extent of illness in the host and the like. Analysis of these factors is well within the skill of one administering immunological treatment. Such individuals would also be capable of determining appropriate dosages based on these factors, as well as through extrapolation of the *in vivo* mouse results presented by Applicants. Moreover, that numerous factors go into the analysis of dosage and time course of administration is well understood in the art. (See, for example, U.S. Patent No. 5,580,859, column 24, lines 10 through 21.)

Finally, the Final Rejection indicates that Applicants' claimed methods should be limited to particle bombardment "since it's the only demonstrated effective method for delivery." This is apparently another concession that effective delivery has been demonstrated notwithstanding the lack of enablement rejection. The Final Rejection apparently relies on Applicants' comment on page 8 of the previous Amendment for the proposition that particle bombardment is "essential" to the claimed methods. It was the Examiner, however, who first brought up the issue of particle bombardment. Specifically, on page 11 of the Office Action dated August 6, 1996, the Office Action concedes that the Nabel reference differs from the present invention in that it does not teach particle

bombardment. The Office Action relies on Eisenbraun as disclosing particle bombardment mediated gene delivery. Applicants' comments, therefore, were in response to the issues raised in the previous Office Action. In any event, Applicants do not believe they advanced the position that particle bombardment was necessary and in fact seemed to argue the opposite. Moreover, Example Section 8 of the originally filed specification clearly provides evidence that subcutaneous injection of particulate polynucleotides is at least as effective as biolistic particulate delivery. (See Example Section 8, particularly page 29, lines 9 through 24.) Thus, Applicants should not be required to limit their claims to particle bombardment.

Rejections Under 35 U.S.C. § 112, Second Paragraph

Claim 59, as amended, was rejected in the Final Rejection under 35 U.S.C. § 112, second paragraph. Applicants have again amended Claim 59. It is submitted that this claim amendment overcomes the rejection under Section 112, second paragraph.

Rejections Under 35 U.S.C. § 103

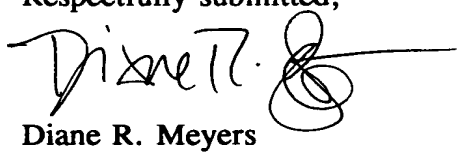
Claims 1 through 67 were initially rejected under 35 U.S.C. § 103 as allegedly being unpatentable over Nabel et al. taken with Eisenbraun et al. and further in view of Robinson et al. Applicants acknowledge with appreciation the withdrawal of this rejection.

SUMMARY

Applicants respectfully submit that clear and convincing data that would be accepted by one skilled in the art has been presented to support enablement of the present invention. Applicants have clearly demonstrated that the methods work in the manner claimed through industry accepted *in vivo* mouse model, and human *in vitro* testing. For all of these reasons, Applicants respectfully

submit that the pending claims are enabled by the specification. Moreover, all of these claims are free from the prior art. A Notice of Allowance is therefore respectfully requested at an early date.

Respectfully submitted,



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